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Derek P. Freyberg — 10/27/03
Derek P. Freyberg — Date

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

In re Application of:

Robert C. Corcoran — Confirmation No.: 8853

App. No.: 09/975,528 — Art Unit: 1723

Filed: 12 October 2001 — Examiner: Ernest G. Therkorn

For: Purification of substances by reaction affinity chromatography

Commissioner for Patents
PO Box 1450
Alexandria VA 22313-1450

Sir:

RESPONSE TO OFFICE ACTION

In response to the Office Action mailed 27 May 2003, for which a two-month extension of the period for response is requested in the accompanying Transmittal and for which the fee is authorized to be paid by credit card in the accompanying PTO-2038, so that the period for response expires on 27 October 2003, please consider the following remarks.

Claims 79-140 are in this application.

The claims were subject to a restriction requirement, and claims 79-81, 87-91, 93, 94, 99, 101, 103-109, 114-116, 121-123, and 128 are under examination; claims 82-86, 92, 95-98, 100, 102, 110-113, 117-120, 124-127, and 129-140 being withdrawn. The restriction requirement was traversed in the response of 14 May 2003, but has been made final.

Claims 79, 80, 87-91, 93, 94, 99, 101, 103-109, 114-116, and 121-123 were rejected under 35 USC 112, ¶1; and all claims under examination were rejected under 35 USC 102(b) and/or 35 USC 103(a). These rejections are respectfully traversed.

The 35 USC 112, ¶1 rejection

Claims 79, 80, 87-91, 93, 94, 99, 101, 103-109, 114-116, and 121-123 were rejected under 35 USC 112, ¶1 as containing new matter, with the Examiner stating that support could not be found in the application as filed for the claim language "without the addition of a reagent acting at the covalent bond." This rejection is respectfully traversed.

Applicant agrees with the Examiner that the application as filed does not provide *ipsis verbis* support for the objected-to language, but submits that: (a) the application as filed is not required to provide *ipsis verbis* support as long as the language finds clear support in the application as filed, and (b) the application as filed does provide such clear support.

First, the reactive affinity chromatography (RAC) method of the invention is always discussed in the application as filed as involving a "naturally reversible reaction" (see for example claim 1 as filed) forming the adduct. Such a reaction is defined in paragraph **[0048]** as follows: "Naturally reversible reaction, as used herein, is a reaction that can reverse itself without the addition of any additional chemical reagents," i.e. it is a reaction that is reversible under the conditions of the contacting, and is also defined in paragraph **[0048]** as being a reaction forming a covalent bond between the reactive functional group (RFG) of the reactive affinity molecule (RAM) and the target to form an adduct. Thus the application provides clear support for the concept that a naturally reversible reaction is one that can reverse itself under the conditions of the contacting without the addition of any additional chemical reagents, and therefore without a reagent acting at the covalent bond, as now expressed in claim 79.

Beyond this, there are two broad cases that can be addressed: those in which the eluent remains constant, and those in which the eluent is changed to modify the affinity of the RAM for the target.

Where the eluent is constant, since the reaction is naturally reversible, it is reversible without the addition of any reagent, and therefore without the addition of a reagent acting at the covalent bond. A more phenomenological justification may be seen at paragraphs **[0038]** and **[0071]**, where a simple version of the RAC method involves recovering the target by simple elution. Since the RAC method inherently involves formation of a covalent bond, and simple elution does not involve the addition of any reagents, "the reaction forming the adduct is reversible under the conditions of the contacting without addition of a reagent acting at the covalent bond," as required by claim 79, and such a reaction is clearly disclosed in the application as filed.

Where the eluent is changed in some fashion to effect a change in the affinity of the reactive functional group for the target, it is clear from the application at paragraphs **[0084]** to **[0090]** that change in properties of the eluent effects a change on the reactivity modifying group, the RMod, rather on the naturally reversible reaction that forms the basis of the RAC process (i.e. the eluent does not act on the covalent bond forming the adduct itself, but on the RMod). Since the RMod is not the same as the RFG or target, nor the adduct resulting from their reaction, the process of bond-forming/bond-breaking occurs without addition of a reagent acting at the covalent bond, and thus also, in the words of claim 79, "the reaction forming the adduct is reversible under the conditions of the contacting without addition of a reagent acting at the covalent bond." Since the RAC method also contemplates the change of an eluent, which is the addition of a reagent that modifies the

RMod, it is clear that the "naturally reversible reaction" of claim 1 as filed is a reaction "reversible under the conditions of the contacting without the addition of a reagent acting at the covalent bond."

Applicant submits that the application as filed, by defining the RAC reaction as naturally reversible and by also permitting the change of an eluent so that there such a reaction may also be influenced by a reagent acting on the RMod, provides clear support for the use in claim 79 of the language "reversible under the conditions of the contacting without the addition of a reagent acting at the covalent bond."

Withdrawal of the rejection is requested.

The 35 USC 102(b)/103(a) rejection over Hylarides

Claims 79, 80, 87-91, 93, 94, 106, 107, 114, 121, and 122 were rejected under 35 USC 102(b) as anticipated by, or under 35 USC 103(a) as obvious over, Hylarides et al., US Patent No. 5,141,646 ("Hylarides"). This rejection is respectfully traversed.

The claimed invention

The invention of the present application, as claimed in claims 79-140, is a method of separating a target from a sample composition containing the target, by:
(a) contacting the sample composition with a reactive affinity molecule attached to a phase separating group, the reactive affinity molecule comprising a reactive functional group, and the reactive affinity molecule reacting with the target to form an adduct by forming a covalent bond between the target and the reactive functional group, where the reaction forming the adduct is reversible under the conditions of the contacting without the addition of a reagent acting at the covalent bond;
(b) separating the adduct from the sample composition;
(c) contacting the adduct with an eluent to regenerate the target and the reactive affinity molecule; and
(d) separating the target from the reactive affinity molecule.

This method, referred to by Applicant as reaction affinity chromatography, relies on the reversible formation of a covalent bond between the target and the reactive functional group of a reactive affinity molecule attached to a phase separating group (e.g. the reversible formation of a covalent bond between the target and a "stationary phase" – to use the language of column chromatography), separation of the adduct from the sample composition (because the adduct is bound to the stationary phase and the remainder of the sample composition is not), the subsequent spontaneous reversal of the covalent bond formation to regenerate the target and the reactive affinity molecule from the adduct (since the reaction is reversible), and the separation of the target from the reactive affinity molecule.

RAC is distinguished from both:
(a) conventional chromatography,
in which *no covalent bond* is formed between the target and the stationary phase (conventional chromatography relies on physical interaction between the target and the stationary phase rather than bond formation),

and the *reaction is reversible*, i.e. the reaction of a target and a stationary phase transiently forms an adduct, but the reaction reverses to regenerate both the target and the stationary phase – so that the stationary phase may be reused for the same separation again and again, and
(b) "covalent chromatography",
in which a *covalent bond is formed* between the target and the stationary phase,
but the reaction forming the covalent bond is *not naturally reversible*, and the covalent bond is broken only by the addition of a reagent acting at the covalent bond, and, though the target is regenerated, the stationary phase is not – so that the stationary phase cannot be reused or can be reused only after a separate chemical regeneration.

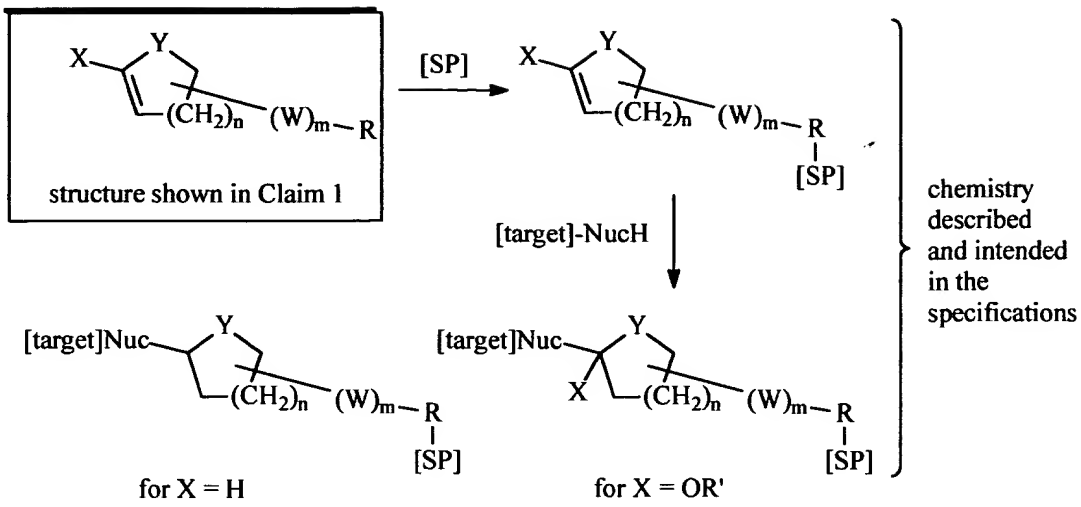
The non-reversibility of the covalent bond formation in covalent chromatography is illustrated, for example, in the excerpt from *Protein purification methods* (reference 10 of the second IDS). This discusses a classic covalent chromatography technique, the isolation of a thiol-containing protein. Figure 36 (page 336) and the text below illustrate the technique. In step (a), the protein (target) interacts with a pyridyl disulfide bound to a support (stationary phase), forming a protein bound to the support by a disulfide linkage (adduct). In step (b), the protein (target) is regenerated by contacting the adduct with an excess of a low molecular weight thiol, but note that the stationary phase is not regenerated in this step, what is left when the protein is removed is a thiol bound to the support. The reaction is not reversed, nor reversible except only in the sense that the target is released: the target is regenerated, but the stationary phase has gone from a pyridyl disulfide to a thiol. Separate treatment in step (c), reacting the immobilized thiol with pyridyl disulfide, is required to regenerate the pyridyl disulfide on the solid support. Other "covalent chromatography" methods employ similarly irreversible reactions.

Thus, while "covalent chromatography" relies on the formation of stable, robust species (e.g. amides, esters, carbamates, disulfides, and acetals) which can only be decomposed by the addition of a reagent acting at the covalent bond, RAC relies on the transient formation of species that are unstable under the conditions of the chromatography. Some of these (hemiacetals, hemiaminals, and the like) are widely recognized as species that cannot be isolated except under special circumstances; others, more apparently stable, contain structural features that cause the adduct to have a transient existence even in the absence of added reagent acting at the covalent bond.

Hylarides

Hylarides discloses a method of isolating a compound by conjugating to a solid phase a reagent that contains a heterocyclic 5- or 6-membered ring with a double bond adjacent the heteroatom (e.g. an N-alkyldihydropyrrole, 2,3-dihydrofuran, 2,3-dihydrothiophene, or analogous 6-membered ring) to form a derivatized solid phase, then contacting that derivatized solid phase with a sample containing a compound with an available nucleophilic group such that the compound bonds to the derivatized solid phase.

This may be illustrated as follows:



The compound is bonded by the formation of a covalent bond between a nucleophilic part of the compound and the double bond of the ring, bonding the compound to the ring at the carbon atom adjacent to the heteroatom. Hylarides states (column 15, lines 47-58) that the covalently bound compound may be released in native form from the solid phase by a variety of ways, such as by mildly acid conditions or by divalent cations, and that this process may be accelerated by heat. "In particular, cleavage occurs by decreasing the pH of a solution contacting the solid phase to pH 6.0 or lower, by adding divalent cations such as Zn^{2+} at a concentration at least equal to that of the reagent attached to the solid phase, or by raising the temperature above 23°C in the presence of pH 6.0 or lower."

Looking at Hylarides Example 3, the sample composition is a mixture of phenethylmercaptoacetamide (the target), S-acetyl-phenethylthioacetamide (the starting material), bis(phenethylaminocarbonylmethyl)disulfide (a byproduct of the hydrolysis of the starting material), and acetic acid (the other hydrolysis byproduct). The reaction mixture is produced with excess hydroxylamine in methanol/water, so the solution is basic. When the solution passes through the column containing the conjugated 5-methyl-2,3-dihydrofuran (the RAM), the target bonds to the furan by formation of a covalent bond to form a conjugated 5-methyl-5-(phenethylaminocarbonyl-methylthio)tetrahydrofuran, a monothioketal. Subsequent lowering of the pH to 4-5 by passing the buffer through the column breaks the S-C bond and regenerates the phenethylmercaptoacetamide and the dihydrofuran.

Discussion

The Examiner apparently (because there is no explicit discussion in this rejection of the section of Hylarides that is being referred to) reasons that the reaction of Hylarides is a reaction that is reversible under the conditions of the contacting without the addition of a reagent acting at the covalent bond (the bond between the target and the RFG). Applicant disagrees.

Hylarides, in Example 3, illustrates contacting at basic pH (because of the use of an excess of hydroxylamine in the hydrolysis reaction producing the mixture to be separated). There is no indication in Hylarides that the reaction forming the S-C bond in the conjugated 5-methyl-5-(phenethylaminocarbonylmethylthio)tetrahydrofuran is reversible at basic pH (i.e. reversible under

the conditions of the contacting); and there is every reason in Hylarides to believe that it is not, because the only method disclosed in Example 3 to release the phenethylmercaptoacetamide is to lower the pH to 4-5. It is stated in Hylarides, at column 15, lines 43-45, that "Following the step of contacting, it may be desirable to wash the solid phase to remove noncovalently bound compounds." This clearly indicates that the reaction forming the S-C bond is not a reaction reversible under the conditions of the contacting, because if it were, washing would not only remove noncovalently bound compounds, it would also act to remove covalently bound material by reversal of the reaction (once any free phenethylmercaptoacetamide is removed by washing, equilibrium inherent in a reversible reaction would cause decomposition of the conjugated 5-methyl-5-(phenethyl-aminocarbonylmethylthio)tetrahydrofuran to release more phenethylmercaptoacetamide, so that washing of the derivatized solid phase under the conditions of the contacting would elute phenethylmercaptoacetamide from the derivatized solid phase rather than just removing noncovalently bound compounds). An illustration that this pH lowering is a direct effect on the atoms/bonds involved in the covalent attachment of the phenethylmercaptoacetamide is that Hylarides discusses elsewhere (e.g. at column 15, lines 46 - 58) not only the reduction of pH to an acid level (below pH 6.0) as a release technique but also as an alternative the use of an excess of a divalent metal cation such as Zn^{2+} – a Lewis acid. It is evident from Hylarides that the reaction between the phenethylmercaptoacetamide and the dihydrofuran is not a reaction that is reversible under the conditions of the contacting without the addition of a reagent acting at the covalent bond.

Looking more generally at Hylarides, it is clear that Hylarides is not talking about reversible reactions because, in every instance, Hylarides discloses that a separate release/cleavage step is needed to recover the target compound (e.g. column 4, lines 9-14 and lines 42-45, column 5, lines 28-36, etc.). The fact that a separate cleavage step is required distinguishes Hylarides from the method of this invention.

By contrast, this invention is limited to the situation in which the reaction between the target and the RFG is one that reversibly forms a covalent bond. For example, paragraph **[0039]** discusses a scheme where thebaine is separated from other opium alkaloids by the use of a nitroso RFG. The nitroso RFG is present on a polymeric resin, which is loaded into a column. The sample composition consisting of thebaine and the other alkaloids is applied to the column, then eluted with a solvent. Because of the reversible covalent bond formation between the thebaine and the nitroso RFG – the reversible formation of a Diels-Alder adduct – passage of the thebaine through the column is delayed, while the other alkaloids, which do not undergo the same reaction, move through the column at the same rate as the eluent.

Applicant submits that Hylarides does not anticipate the claims because Hylarides does not disclose a process in which an adduct is formed by a covalent bond-forming reaction that is reversible under the conditions of the contacting without the addition of a reagent acting at the covalent bond. A rejection for anticipation will only stand if the reference discloses every element of the claim, and Hylarides fails to disclose that reversibility of the reaction.

Further, Applicant submits that Hylarides does not render the claims obvious because there is neither disclosure nor suggestion in Hylarides that the covalent bond-forming reaction should be anything other than a reaction that *requires* the addition of a reaction acting at the covalent bond for reversal. Indeed, a fair reading of Hylarides suggests exactly the contrary: a reversible reaction would be undesirable because washing of the solid phase would reverse the reaction and lose the

target material. A rejection for obviousness will only stand if the reference suggests the modification and its likelihood of success, and Hylarides offers neither suggestion of a reaction reversible without the addition of a reagent acting at the covalent bond, nor any expectation of success if such a reaction were employed.

Withdrawal of the rejection is requested.

The 35 USC 103(a) rejection over Hylarides in view of Schössler or Carron and Sohar

Claims 81, 123, and 128 were rejected under 35 USC 103(a) as being unpatentable over Hylarides in view of either Schössler et al., US Patent No. 4,822,681 ("Schössler") or Carron et al., PCT Publication No. WO 98/59234 ("Carron") and Sohar et al., US Patent No. 3,894,026 ("Sohar"). This rejection is respectfully traversed.

The Examiner asserts that "at best, the claims differ from Hylarides in reciting use of a nitroso group and targeting a 1,3-diene group" and that the secondary references fill this gap. Applicant disagrees.

With respect to Schössler, the Examiner states that Schössler (column 3, lines 20-22) discloses that a nitroso group is interchangeable with Hylarides' (column 32, lines 60-63) amino, carbonyl, and sulfhydryl groups. Applicants accept that Schössler discloses the formation of polymer solid body surfaces with functionalized silyl groups and that these silyl groups may be functionalized with, *inter alia*, nitroso groups as well as amino, carbonyl, and sulfhydryl groups. However, these surface groups are by no means the equivalent of the groups disclosed at column 32, lines 60-63, of Hylarides. The groups R claimed at column 32, lines 60-63 of Hylarides are not the reactive functional groups of a reactive affinity molecule, they are the means by which Hylarides' dihydrofuran or other heterocycle (the reactive portion of the molecule) is attached to the solid phase support. *See* Hylarides at column 15, lines 34-36: "The step of conjugating the reagent to the solid phase attaches the former to the latter via R, thereby forming a derivatized solid phase," and *see* throughout Hylarides, where it is made clear that the bonding of the "target" occurs by covalent bonding to the carbon atom adjacent the heteroatom of the heterocycle. Referring to Example 3 of Hylarides, the dihydrofuran is attached through an acetic acid to an N-hydroxysulfosuccinimidate, and it is this portion of the molecule that reacts with the aminohexyl Sepharose to link the dihydrofuran to the resin. It is the dihydrofuran that reacts with the phenethylmercaptoacetamide, not the sulfosuccinimidate. Thus Hylarides' amino, carbonyl and sulfhydryl R groups are not groups to which a nitroso group of Schössler is analogous, they are if anything analogous to the alkoxy, phenoxy, or halogen R groups of Schössler.

Further, although Schössler discloses the functionalizing of the surfaces of polymer bodies with groups including nitroso groups, there is no suggestion in Schössler that the reactions of these functional groups with target molecules should be reversible without the addition of a reagent acting at the covalent bond. In fact, Schössler is to the contrary: Schössler's general discussion is of the formation of surfaces "upon which proteins, nucleic acids, low-molecular ligands, cells, microorganisms and other biological materials can be bound with *high stability* and yield, as well as biocompatibility" (column 2, lines 65-68, emphasis added); and Schössler's discussion of reversibility (column 5, lines 3-12) refers to the formation of disulfide groups which can be cleaved "by means of the employment of suitable reducing agent," clearly illustrating bonding that is not

reversible without the addition of a reagent acting at the covalent bond. Further, there is nothing in either Hylarides or Schössler suggesting the desirability of the proposed combination.

Thus Schössler fails to remedy the deficiency of Hylarides in not showing a nitroso group as reactive functional group and 1,3-diene as target, and the combination does not meet the claims.

With respect to Carron, the argument is the same as for Schössler. While Carron discloses a variety of reactive functional groups (including the nitroso group mentioned by the Examiner) that may interact with analytes, these groups – precisely because they do interact with the analytes – are not analogous to the carbonyl R group of Hylarides, which is a group bonding to the solid phase. Further, there is nothing in either Hylarides or Carron suggesting the desirability of the proposed combination.

Thus Carron also fails to remedy the deficiencies of Hylarides, and the combination does not meet the claims.

The Examiner cites Sohar as disclosing the chromatographic purification of thebaine, a 1,3-diene. However, Sohar discloses only conventional techniques and does not disclose the use of methods involving reversible formation of covalent bonds. For example, the Examiner refers to column 4, lines 25-28, disclosing liquid chromatography of thebaine to assay purity. No details are given at that location, but column 3, lines 48-51 discusses the use of a 2 IPAX column coated with 2-cyanoethyl ether and eluting with 10% ethanol/hexane 25% saturated with 2-cyanoethyl ether. Such a support is not known or expected to react with thebaine or 1,3-dienes at all, much less reversibly: chromatographic media of this type are known to act by the physical partitioning of a substance between a liquid-like stationary phase and a liquid mobile phase. The other method cited (column 4, lines 55-57) involves alumina, to which no reversible or non-reversible reaction with thebaine or other 1,3-dienes is expected: chromatography on alumina involves absorption/desorption to the alumina. No specificity associated with chemical reaction is expect or found; and of course Sohar does not mention nitroso groups or reversible covalent bonding. Further, there is nothing in any of Hylarides, Schössler, Carron, or Sohar suggesting the desirability of the proposed combination.

Thus Sohar fails to remedy the deficiencies of both Hylarides in view of Schössler and Hylarides in view of Carron, and the combination does not meet the claims. Withdrawal of the rejection is requested.

The 35 USC 103(a) rejection over Hylarides in view of Stevens and Schössler

Claim 91 was rejected under 35 USC 103(a) as being unpatentable over Hylarides in view of Stevens et al, US Patent No. 4,927,539 ("Stevens") and Schössler. This rejection is respectfully traversed.

The Examiner asserts that "at best, the claims differ from Hylarides in reciting use of a macroreticular polymer", and that Stevens discloses that a macroporous polymer has a higher capacity and Schössler discloses that reactive supports are conventionally macroporous, so that it would have been obvious to use a macroreticular polymer in the method of Hylarides, making the claim obvious. Applicant disagrees.

Applicant agrees that macroreticular polymers have a higher capacity than non-macroporous polymers and that Schössler discloses that reactive supports are frequently macroporous. However, neither Stevens nor Schössler, alone or in combination, remedy the deficiencies of Hylarides in failing to disclose or suggest that the method of the present invention involve a reaction that is reversible under the conditions of the contacting without the addition of a reagent acting at the covalent bond; so that the combination of Hylarides and Stevens (and Schössler) fails to meet the claim. Withdrawal of the rejection is requested.

The 35 USC 103(a) rejection over Hylarides in view of Carron or Duran

Claims 99, 101, and 103-105 were rejected under 35 USC 103(a) as being unpatentable over Hylarides in view of Carron or Duran et al., PCT International Publication No. WO 99/16907 ("Duran"). This rejection is respectfully traversed.

The Examiner asserts that "at best, the claims differ from Hylarides in reciting use of a reactivity modifier group", that Carron "discloses modifiers such as amines influence the reactivity between the reactive functional group and the analyte", and that Duran "discloses ionic compounds such as amines attract target molecules", so that it would be obvious to use a modifier in Hylarides. Applicant disagrees.

Applicant agrees that Carron discloses that the reactivity of reactive functional groups can be modified by the use of reactivity modifier groups; however Carron fails to remedy the previously-mentioned basic deficiency of Hylarides in failing to disclose or suggest that the method of the present invention involve a reaction that is reversible under the conditions of the contacting without the addition of a reagent acting at the covalent bond. Applicant agrees that Duran discloses the use of charged groups, but these charged groups are put on a surface in order to attract target molecules through ion-pairing effects. *See* Duran at page 4, lines 18-19, where the attractive force is "between attractive (e.g. ionic) groups on the bound reagent and oppositely charged groups on the target. Once attracted to the bound reagent, and in turn to the surface, the target molecule can be thermochemically coupled to the bound reagent by reaction between the reactive groups of the bound reagent and appropriate functional groups on the target molecule. The thermochemically reactive groups and the ionic groups can either be on the same polymer or on different polymers that are coimmobilized on the surface." Thus, Duran's ionic groups are not reactivity modifiers, because they are used solely for ionic attraction and because they can be on different polymers, and the sections cited by the Examiner are not to the contrary - indeed, they emphasize this very point. Also, in the purification of thebaine (paragraphs **[0039]** and **[00116]** and Figure 5), affinity of the thebaine for the reactive functional group is increased when the reactivity modifier group and the thebaine are both positively charged, which is directly contradictory to the effect of Duran. Further, Duran relates to a method for irreversible covalent attachment of oligos to surfaces. Applicant submits that there is no motivation in either Hylarides or Duran for the combination proposed by the Examiner and that, even if such a combination were made, it would not meet the claims because Duran fails to remedy the deficiency of Hylarides in failing to disclose or suggest that the method of the present invention involve a reaction that is reversible under the conditions of the contacting without the addition of a reagent acting at the covalent bond and because Duran does not disclose reactivity modifier groups. Withdrawal of the rejections is requested.

The 35 USC 103(a) rejection over Hylarides in view of Schössler or Carron

Claims 108 and 109 were rejected under 35 USC 103(a) as being unpatentable over Hylarides in view of either Schössler or Carron. This rejection is respectfully traversed.

The Examiner asserts that "at best, the claims differ from Hylarides in reciting use of a nitroso group", that Schössler discloses that a nitroso group is interchangeable with Hylarides' amino, sulfhydryl, or carbonyl groups, and that Carron discloses that a nitroso group is interchangeable with Hylarides' carbonyl group, so that it would be obvious to use a nitroso group in Hylarides. Applicant disagrees.

The argument with respect to these claims is the same as the argument with respect to claims 81, 123, and 128 discussed at pages 6 and 7.

Applicant accepts that Schössler discloses the formation of polymer solid body surfaces with functionalized silyl groups and that these silyl groups may be functionalized with, *inter alia*, nitroso groups as well as amino, carbonyl, and sulfhydryl groups. However, Hylarides' amino, carbonyl and sulfhydryl R groups are not groups to which a nitroso group of Schössler is analogous, they are if anything analogous to the alkoxy, phenoxy, or halogen R groups of Schössler. Also, there is no suggestion in Schössler that the reactions of these functional groups with target molecules should be reversible without the addition of a reagent acting at the covalent bond. Further, there is nothing in either Hylarides or Schössler suggesting the desirability of the proposed combination. With respect to Carron, the argument is the same as for Schössler. While Carron discloses a variety of reactive functional groups (including the nitroso group mentioned by the Examiner) that may interact with analytes, these groups – precisely because they do interact with the analytes – are not analogous to the carbonyl R group of Hylarides, which is a group bonding to the solid phase. Further, there is nothing in either Hylarides or Carron suggesting the desirability of the proposed combination. *See* the previous discussion for detail, which is omitted here in the interest of brevity. Thus neither Schössler nor Carron is combinable with Hylarides, and the combinations proposed fail to meet the claims. Withdrawal of the rejections is requested.

The 35 USC 103(a) rejection over Hylarides in view of Kohn

Claims 79, 80, 87-91, 93-94, 106, 107, 114-116, 121, and 122 were rejected [two separate rejections were made on pages 5-6 and 6, but the reasons given were identical and the second encompassed the first, so only one response is made] under 35 USC 103(a) as being unpatentable over Hylarides in view of Kohn et al., US Patent No. 6,362,008 ("Kohn"). This rejection is respectfully traversed.

The Examiner asserts that "at best, the claims differ from Hylarides in reciting use of methanol as an eluent", and that Kohn discloses that the use of methanol is a known releasing agent for covalent chromatography, so that it would be obvious to use methanol in Hylarides. Applicant disagrees.

Applicants accepts that Kohn, column 7, lines 59-62, says that "The use of covalent chromatography is similar to other affinity chromatography procedures", but notes that it says nothing about methanol. Column 7, lines 16-25 also says nothing about methanol. If the Examiner intended to refer to column 12, lines 16-25, Applicant agrees that the section suggests that methanol

is disclosed as one of three possible solvents for the release of bound T-2 from antibodies on a matrix, but this is not covalent chromatography, it is antibody-analyte affinity chromatography, which is widely recognized not to involve covalent bonding. As a person of skill in the art would recognize, high concentrations of methanol, ethanol, or acetonitrile denature most proteins, including antibodies, so their use as release agents for affinity chromatography is understandable. However, there is nothing in Kohn that suggests that methanol would be useful as an eluent for covalent chromatography, still less that it would be useful as an eluent for the reaction affinity chromatography of this invention. Applicants note that the resin used for the separation of column 12, lines 16-25 is not a matrix of the type disclosed in column 7, such as a thiol-containing matrix, it is an activated Sepharose 4B gel, which is a simple gel-permeation chromatography medium (and indeed Kohn suggests that the antibodies can be bound to matrices as simple as a glass plate, *see* column 12, line 24). Further, there is nothing in Hylarides or Kohn to suggest the desirability of the combination – Hylarides performs his covalent chromatography separations with acid or divalent metal ion Lewis acid, and Kohn his affinity chromatography separations with an antibody-denaturing solvent such as methanol, ethanol, or acetonitrile; and there is nothing in either reference to suggest the substitution of methanol for Hylarides' reagents (and every reason from the art to believe that the substitution would not be useful). Finally, the combination does not remedy the deficiency of Hylarides in failing to disclose or suggest that the method of the present invention involve a reaction that is reversible under the conditions of the contacting without the addition of a reagent acting at the covalent bond. Withdrawal of the rejections is requested.

The 35 USC 103(a) rejection over Hylarides in view of Kohn and Schössler or Carron and Sohar

Claims 81, 123, and 128 were rejected under 35 USC 103(a) as being unpatentable over Hylarides in view of Kohn further in view of either Schössler or Carron and Sohar. This rejection is respectfully traversed.

The Examiner asserts that "at best, the claims differ from Hylarides and Kohn in reciting use of a nitroso group and targeting a 1,3-diene group" and that the secondary references fill this gap. Applicant disagrees.

As discussed above, there is nothing in Hylarides or Kohn to suggest the desirability of the combination proposed – the two methods are quite different, covalent chromatography released with protic acid or Lewis acid, and affinity chromatography released with an antibody-denaturing agent such as an alcohol or acetonitrile, and neither discloses a covalent bond-forming reaction reversible under the conditions of the contacting without the addition of a reagent acting at the covalent bond.

The deficiencies of the proposed combination of Hylarides with Schössler or Carron and Sohar have been discussed before at pages 6-8, and the argument here is the same, because Kohn (for the reasons discussed above with respect to the rejection over Hylarides and Kohn alone) adds nothing to Hylarides.

Applicant accepts that Schössler discloses the formation of polymer solid body surfaces with functionalized silyl groups and that these silyl groups may be functionalized with, *inter alia*, nitroso groups as well as amino, carbonyl, and sulfhydryl groups. However, Hylarides' amino, carbonyl and sulfhydryl R groups are not groups to which a nitroso group of Schössler is analogous, they are if anything analogous to the alkoxy, phenoxy, or halogen R groups of Schössler. Also, there is no

suggestion in Schössler that the reactions of these functional groups with target molecules should be reversible without the addition of a reagent acting at the covalent bond. Further, there is nothing in either Hylarides or Schössler suggesting the desirability of the proposed combination. With respect to Carron, the argument is the same as for Schössler. While Carron discloses a variety of reactive functional groups (including the nitroso group mentioned by the Examiner) that may interact with analytes, these groups – precisely because they do interact with the analytes – are not analogous to the carbonyl R group of Hylarides, which is a group bonding to the solid phase. Further, there is nothing in either Hylarides or Carron suggesting the desirability of the proposed combination. *See* the previous discussion for detail, which is omitted here in the interest of brevity. Thus neither Schössler nor Carron is combinable with Hylarides, and the combinations proposed fail to meet the claims. While Sohar discloses the chromatographic purification of thebaine, a 1,3-diene, Sohar discloses only conventional techniques and does not disclose the use of methods involving reversible formation of covalent bonds. No specificity associated with chemical reaction is expect or found; and of course Sohar does not mention nitroso groups or reversible covalent bonding. Further, there is nothing in any of Hylarides, Schössler, Carron, or Sohar suggesting the desirability of the proposed combination. Thus Sohar fails to remedy the deficiencies of both Hylarides in view of Schössler and Hylarides in view of Carron, and the combination does not meet the claims. These deficiencies are not remedied by the addition of Kohn, because Kohn relates to affinity chromatography, not involving formation of covalent bonds, and therefore (a) is not combinable because the techniques are non-analogous and the proposed solvent substitution of the combination is non-functional, and (b) still fails to meet the claims because none of the documents remedy the basic deficiency of Hylarides in failing to disclose or suggest that the method of the present invention involve a reaction that is reversible under the conditions of the contacting without the addition of a reagent acting at the covalent bond. Withdrawal of the rejection is requested.

The 35 USC 103(a) rejection over Hylarides in view of Kohn and Stevens and Schössler

Claim 91 was rejected under 35 USC 103(a) as being unpatentable over Hylarides in view of Stevens et al, US Patent No. 4,927,539 ("Stevens") and Schössler. This rejection is respectfully traversed.

The Examiner asserts that "at best, the claims differ from Hylarides in view of Kohn in reciting use of a macroreticular polymer", and that Stevens discloses that a macroporous polymer has a higher capacity and Schössler discloses that reactive supports are conventionally macroporous, so that it would have been obvious to use a macroreticular polymer in the method of Hylarides and Kohn, making the claim obvious. Applicant disagrees.

The deficiencies of the combination of Hylarides and Kohn have been pointed out previously at page 10, and that argument is incorporated here by reference for brevity. In addition to these deficiencies, neither Stevens nor Schössler, alone or in combination, remedy the deficiencies of Hylarides and Kohn (assuming that the combination could properly be made) in failing to disclose or suggest that the method of the present invention involve a covalent bond-forming reaction that is reversible under the conditions of the contacting without the addition of a reagent acting at the covalent bond; so that the combination of Hylarides, Kohn, and Stevens (and Schössler) fails to meet the claim. Withdrawal of the rejection is requested.

The 35 USC 103(a) rejection over Hylarides in view of Kohn and Carron or Duran

Claims 99, 101, and 103-105 were rejected under 35 USC 103(a) as being unpatentable over Hylarides in view of Kohn further in view of Carron or Duran. This rejection is respectfully traversed.

The Examiner asserts that "at best, the claims differ from Hylarides in view of Kohn in reciting use of a reactivity modifier group", that Carron "discloses modifiers such as amines influence the reactivity between the reactive functional group and the analyte", and that Duran "discloses ionic compounds such as amines attract target molecules", so that it would be obvious to use a modifier in Hylarides. Applicant disagrees.

As discussed above, there is nothing in Hylarides or Kohn to suggest the desirability of the combination proposed – the two methods are quite different, covalent chromatography released with protic acid or Lewis acid, and affinity chromatography released with an antibody-denaturing agent such as an alcohol or acetonitrile, and neither discloses a reaction reversible under the conditions of the contacting without the addition of a reagent acting at the covalent bond.

The deficiencies of the proposed combination of Hylarides with Carron or Duran have been discussed before at pages 8-9, and the argument here is the same, because Kohn (for the reasons discussed above with respect to the rejection over Hylarides and Kohn alone) adds nothing to Hylarides.

Carron fails to remedy the previously-mentioned basic deficiency of Hylarides in failing to disclose or suggest that the method of the present invention involve a reaction that is reversible under the conditions of the contacting without the addition of a reagent acting at the covalent bond. Duran discloses the use of charged groups, but these charged groups are put on a surface in order to attract target molecules through ion-pairing effects and are not reactivity modifiers, and Duran relates to a method for irreversible covalent attachment of oligos to surfaces. Applicant submits that there is no motivation in either Hylarides and Carron or Duran for the combination proposed by the Examiner and that, even if such a combination were made, it would not meet the claims.

The deficiencies of the combination of Hylarides and Kohn have been pointed out previously at page 10, and that argument is incorporated here by reference for brevity. In addition to these deficiencies, neither Carron nor Duran, alone or in combination, remedy the deficiencies of Hylarides and Kohn (assuming that the combination could properly be made; so that the combination of Hylarides, Kohn, and Carron or Duran fails to meet the claims. Withdrawal of the rejection is requested.

The 35 USC 103(a) rejection over Hylarides and Kohn in view of Schössler or Carron

Claims 108 and 109 were rejected under 35 USC 103(a) as being unpatentable over Hylarides in view of Kohn further in view of either Schössler or Carron. This rejection is respectfully traversed.

The Examiner asserts that "at best, the claims differ from Hylarides in view of Kohn in reciting use of a nitroso group", that Schössler discloses that a nitroso group is interchangeable with Hylarides' amino, sulfhydryl, or carbonyl groups, and that Carron discloses that a nitroso group is

interchangeable with Hylarides' carbonyl group, so that it would be obvious to use a nitroso group in Hylarides. Applicant disagrees.

As discussed above, there is nothing in Hylarides or Kohn to suggest the desirability of the combination proposed – the two methods are quite different, covalent chromatography released with protic acid or Lewis acid, and affinity chromatography released with an antibody-denaturing agent such as an alcohol or acetonitrile, and neither discloses a reaction reversible under the conditions of the contacting without the addition of a reagent acting at the covalent bond.

The deficiencies of the proposed combination of Hylarides with Schössler or Carron have been discussed before at pages 6-7 and 9, and the argument here is the same, because Kohn (for the reasons discussed above with respect to the rejection over Hylarides and Kohn alone) adds nothing to Hylarides.

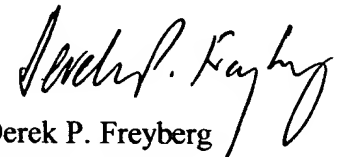
Hylarides' amino, carbonyl and sulfhydryl R groups are not groups to which a nitroso group of Schössler is analogous, they are if anything analogous to the alkoxy, phenoxy, or halogen R groups of Schössler; there is no suggestion in Schössler that the reactions of these functional groups with target molecules should be reversible without the addition of a reagent acting at the covalent bond; and there is nothing in either Hylarides or Schössler suggesting the desirability of the proposed combination. While Carron discloses a variety of reactive functional groups (including the nitroso group mentioned by the Examiner) that may interact with analytes, these groups – precisely because they do interact with the analytes – are not analogous to the carbonyl R group of Hylarides; and there is nothing in either Hylarides or Carron suggesting the desirability of the proposed combination. Thus neither Schössler nor Carron is combinable with Hylarides, and the combinations proposed fail to meet the claims.

The deficiencies of the combination of Hylarides and Kohn have been pointed out previously at page 10, and that argument is incorporated here by reference for brevity. In addition to these deficiencies, neither Schössler nor Carron, alone or in combination, remedy the deficiencies of Hylarides and Kohn (assuming that the combination could properly be made); so that the combination of Hylarides, Kohn, and Carron or Duran fails to meet the claims. Withdrawal of the rejection is requested.

Conclusi n

Applicant respectfully submits that claims 79, 80, 87-91, 93, 94, 99, 101, 103-109, 114-116, and 121-123 comply with 35 USC 112, ¶1 and are not drawn to new matter, and that none of claims 79-81, 87-91, 93, 94, 99, 101, 103-109, 114-116, 121-123, and 128 are either anticipated by or obvious over the references and combinations of references cited by the Examiner. Withdrawal of the rejections, examination of the non-elected claims, and allowance of the application are respectfully requested.

Respectfully submitted,



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